

(i) selecting a first candidate gene;

(ii) providing a first zinc finger protein that binds to a first target site of the first candidate gene and a second zinc finger protein that binds to a target site of a second gene;

gene;

(iii) culturing a first cell under conditions where the first zinc finger protein contacts the first candidate gene and culturing a second cell under conditions where the second zinc finger protein contacts the second candidate gene, wherein the first and the second zinc finger proteins modulate expression of the first and second candidate genes; and

identifying whether or not

genes; and

(iv) assaying for a selected phenotype, thereby identifying whether or not the first candidate gene is associated with the selected phenotype.

2. The method of claim 1, further comprising providing a third zinc finger protein that binds to a second target site of the first candidate gene.

3. The method of claim 1, further comprising selecting a plurality of candidate genes and providing a plurality of zinc finger proteins that bind to a target site of each candidate gene.

4. The method of claim 1, wherein the second gene is a control gene.

5. The method of claim 1, wherein the first candidate gene is partially encoded by an EST of at least about 200 nucleotides in length.

6. The method of claim 1, wherein the first candidate gene and the second gene are both associated with the selected phenotype.

7. The method of claim 1, wherein the first and second cell are the same cell, wherein the cell comprises the first and second candidate genes.

8. The method of claim 1, wherein the first and the second candidate genes are endogenous genes.

Sub A48
1 9. The method of claim 1, wherein expression of the candidate genes
2 is inhibited by at least about 50%.

1 10. The method of claim 1, wherein expression of the candidate genes
2 is activated by at least about 150%.

1 11. The method of claim 1, wherein the zinc finger proteins are fusion
2 proteins comprising a regulatory domain.

Sub A5
1 12. The method of claim 1, wherein expression of the zinc finger
2 proteins is induced by administration of an exogenous agent.

1 13. The method of claim 11, wherein the zinc finger proteins are fusion
2 proteins comprising at least two regulatory domains.

1 14. The method of claim 1, wherein the cell is selected from the group
2 consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.

1 15. The method of claim 14, wherein the cell is a mammalian cell

1 16. The method of claim 15, wherein the cell is a human cell

1 17. The method of claim 1, wherein the modulation of expression is
2 activation of gene expression that prevents repression of gene expression.

1 18. The method of claim 1, wherein the modulation of expression is
2 inhibition of gene expression that prevents gene activation.

1 19. The method of claim 11, wherein the regulatory domain is selected
2 from the group consisting of a transcriptional repressor, a methyl transferase, a
3 transcriptional activator, a histone acetyltransferase, and a histone deacetylase.

Sub A6
1 20. The method of claim 1, wherein the first and second zinc finger
2 proteins are encoded by an expression vector comprising a zinc finger protein nucleic
3 acid operably linked to a promoter, and wherein the method further comprises the step of
4 first administering the expression vector to the cell.

1 21. The method of claim 20, wherein expression of the zinc finger
2 proteins is under small molecule control.

Sub A6
1 22. The method of claim 21, wherein expression of the first zinc finger
2 protein and expression of the second zinc finger protein are under different small
3 molecule control, wherein both the first and the second zinc finger protein are fusion
4 proteins comprising a regulatory domain, and wherein the first and the second zinc finger
5 proteins are expressed in the same cell.

1 23. The method of claim 22, wherein both the first and the second zinc
2 finger proteins comprise a regulatory domain that represses gene expression.

1 24. The method of claim 20, wherein the expression vector is a viral
2 vector.

1 25. The method of claim 24, wherein the expression vector is a
2 retroviral expression vector, an adenoviral expression vector, or an AAV expression
3 vector.

1 26. The method of claim 20, wherein the zinc finger proteins are
2 encoded by a nucleic acid operably linked to an inducible promoter.

1 27. The method of claim 1, wherein the cell comprises less than about
2 1.5×10^6 copies of each zinc finger protein.

1 28. The method of claim 1, wherein the target site is upstream of a
2 transcription initiation site of the candidate gene.

Sub A7
1 29. The method of claim 1, wherein the target site is adjacent to a
2 transcription initiation site of the candidate gene.

1 30. The method of claim 1, wherein the target site is adjacent to an
2 RNA polymerase pause site downstream of a transcription initiation site of the candidate
3 gene.

1 31. A method of identifying the biological function of a candidate
2 gene, the method comprising the steps of:

- 3 (i) identifying a plurality of candidate genes;
 4 (ii) providing a first zinc finger protein that binds to a first target site of a
 5 first candidate gene;
 6 (iii) culturing a first cell under conditions where the first zinc finger
 7 protein contacts the first candidate gene, wherein the first zinc finger protein modulates
 8 expression of the first candidate gene;
 9 (iv) determining the expression pattern of the candidate genes and
 10 determining whether or not the first candidate gene is associated with the selected
 11 phenotype; and
 12 (v) repeating steps (ii)-(iv) for each candidate gene.

1 32. The method of claim 31, further comprising providing a second
 2 zinc finger protein that binds to a second target site of the first candidate gene.

1 33. The method of claim 31, wherein at least one of the candidate
 2 genes is an EST of at least about 200 nucleotides in length.

1 34. The method of claim 31, wherein at least two candidate genes are
 2 required to cause the selected phenotype.

1 35. The method of claim 31, wherein the candidate genes are
 2 endogenous genes.

1 36. The method of claim 31, wherein expression of the candidate genes
 2 is inhibited by at least about 50%.

1 37. The method of claim 31, wherein expression of the candidate genes
 2 is activated to at least about 150%.

1 38. The method of claim 31, wherein the zinc finger protein is a fusion
 2 protein comprising a regulatory domain.

1 39. The method of claim 38, wherein the regulatory domain is under
 2 small molecule control.

1 40. The method of claim 38, wherein the zinc finger proteins are fusion
 2 proteins comprising at least two regulatory domains.

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1 41. The method of claim 31, wherein the cell is selected from the
2 group consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal
3 cell.

1 42. The method of claim 41, wherein the cell is a mammalian cell

1 43. The method of claim 42, wherein the cell is a human cell

1 44. The method of claim 31, wherein the modulation of expression is
2 activation of gene expression that prevents repression of gene expression.

1 45. The method of claim 31, wherein the modulation of expression is
2 inhibition of gene expression that prevents gene activation.

1 46. The method of claim 38, wherein the regulatory domain is selected
2 from the group consisting of a transcriptional repressor, a methyl transferase, a
3 transcriptional activator, a histone acetyltransferase, and a histone deacetylase.

1 47. The method of claim 31, wherein the zinc finger protein is encoded
2 by an expression vector comprising a zinc finger protein nucleic acid operably linked to a
3 promoter, and wherein the method further comprises the step of first administering the
4 expression vector to the cell.

1 48. The method of claim 47, wherein expression of the zinc finger
2 protein is under small molecule control.

1 49. The method of claim 47, wherein the expression vector is a viral
2 vector.

1 50. The method of claim 49, wherein the expression vector is a
2 retroviral expression vector, an adenoviral expression vector, or an AAV expression
3 vector.

1 51. The method of claim 47, wherein the zinc finger protein is encoded
2 by a nucleic acid operably linked to an inducible promoter.

1 52. The method of claim 31, wherein the cell comprises less than about
2 1.5×10^6 copies of the zinc finger protein.

1 53. The method of claim 31, wherein the target site is upstream of a
2 transcription initiation site of the candidate gene.

1 54. The method of claim 31, wherein the target site is adjacent to a
2 transcription initiation site of the candidate gene.

1 55. The method of claim 31, wherein the target site is adjacent to an
2 RNA polymerase pause site downstream of a transcription initiation site of the candidate
3 gene.

1 56. A method of identifying the biological function of a candidate
2 gene, the method comprising the steps of:

3 (i) selecting a first candidate gene;

4 (ii) providing a first zinc finger that binds to a first target site of the first
5 candidate gene and a second zinc finger that binds to a second target site of the first
6 candidate gene;

7 (iii) culturing a first cell under conditions where the first zinc finger
8 protein contacts the first candidate gene, and culturing a second cell under conditions
9 where the second zinc finger protein contacts the first candidate gene, wherein the first
10 and the second zinc finger proteins modulate expression of the first candidate gene; and

11 (iv) assaying for a selected phenotype, thereby identifying whether or not
12 the first candidate gene is associated with the selected phenotype.

1 57. The method of claim 56, further comprising providing a third zinc
2 finger protein that binds to a target site of a second candidate gene.

1 58. The method of claim 56, further comprising selecting a plurality of
2 candidate genes and providing a plurality of zinc finger proteins that bind to a target site
3 of each candidate gene.

1 59. The method of claim 57, wherein the second candidate gene is a
2 control gene.

1 60. The method of claim 56, wherein the first candidate gene is an EST
2 of at least about 200 nucleotides in length.

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61. The method of claim 57, wherein the first candidate gene and the second candidate gene are both required for causing the selected phenotype.
62. The method of claim 56, wherein the first and second cell are the same cell.
63. The method of claim 56, wherein the first candidate gene is an endogenous gene.
64. The method of claim 56, wherein expression of the first candidate gene is inhibited by at least about 50%.
65. The method of claim 56, wherein expression of the first candidate gene is activated to at least about 150%.
66. The method of claim 56, wherein the first zinc finger protein is a fusion protein comprising a regulatory domain.
67. The method of claim 66, wherein the regulatory domain is under small molecule control.
68. The method of claim 66, wherein the zinc finger proteins are fusion proteins comprising at least two regulatory domains.
69. The method of claim 56, wherein the cell is selected from the group consisting of animal cell, a plant cell, a bacterial cell, a protozoal cell, or a fungal cell.
70. The method of claim 69, wherein the cell is a mammalian cell.
71. The method of claim 71, wherein the cell is a human cell.
72. The method of claim 56, wherein the modulation of expression is activation of gene expression that prevents repression of gene expression.
73. The method of claim 56, wherein the modulation of expression is inhibition of gene expression that prevents gene activation.

75. The method of claim 56, wherein the first and the second zinc finger proteins are encoded by an expression vector comprising a zinc finger protein nucleic acid operably linked to a promoter, and wherein the method further comprises the step of first administering the expression vector to the cell.

2 proteins is under small molecule control.

1 77. The method of claim 76, wherein expression of the first zinc finger

2 protein and expression of the second zinc finger protein are under different small

3 molecule control, wherein both the first and the second zinc finger protein are fusion

4 proteins comprising a regulatory domain, and wherein the first and the second zinc finger

5 proteins are expressed in the same cell.

3 protein comprises a region of the protein that is located in the cytoplasm of the cell.

1 79. The method of claim 75, wherein the expression vector is a viral

2 vector.

81. The method of claim 75, wherein the zinc finger proteins are encoded by a nucleic acid operably linked to an inducible promoter.

83. The method of claim 56, wherein the first or the second target site is upstream of a transcription initiation site of the first candidate gene.

1 85. The method of claim 56, wherein the first or the second target site
2 is adjacent to an RNA polymerase pause site downstream of a transcription initiation site
3 of the first candidate gene.

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